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Hybridization Experiments in Acheilognathine Fishes (Cyprinidae, Teleostei). A Comparison of the Intergeneric Hybrids between *Tanakia tanago* and *Rhodeus spinalis* and *Rhodeus ocellatus* from Korea and Japan

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(Plates I & II; Text-figure 1)

In a recent paper the present author (Duyvené de Wit, in press) has summarized the results of his investigations into the presence of gametic compatibility between a number of acheilognathine[1] species.

From Text-fig. 1, in which the ability of these species to cross is represented diagramatically, it appears that gametic compatibility is not limited to the subspecific and specific levels, but also manifests itself on the generic level. In the majority of the intergeneric crosses, the F_1 offspring consisted of phenotypical males only, but the intergeneric hybrids between the allopatric species *Tanakia tanago* (Tanaka) from Japan and *Acheilognathus himantegus* (Günther) from Taiwan consisted of fertile males and females. These fishes were allowed to interbreed freely in the presence of freshwater mussels and this experimental interbreeding population has now been carried through its fourth generation. The phenotypic character of all four generations has remained stable.

In another paper (Duyvené de Wit, 1961), we have expressed the view that *Rhodeus spinalis* (Oshima) from Taiwan and the two strains of *Rhodeus ocellatus* (Kner), which have their habitats in Japan and Korea respectively, represent three allopatric geographic subspecies belonging to a single species. As will be seen from Text-fig. 1, the hybrids produced from these strains consisted of fertile males and females.

[1]Following Chu (1935) and Hubbs & Kuronuma (1943), the designation Acheilognathinae instead of Rhodeinae is used, because *Acheilognathus* (Bleeker, 1863) has priority over *Rhodeus* (Günther, 1868).

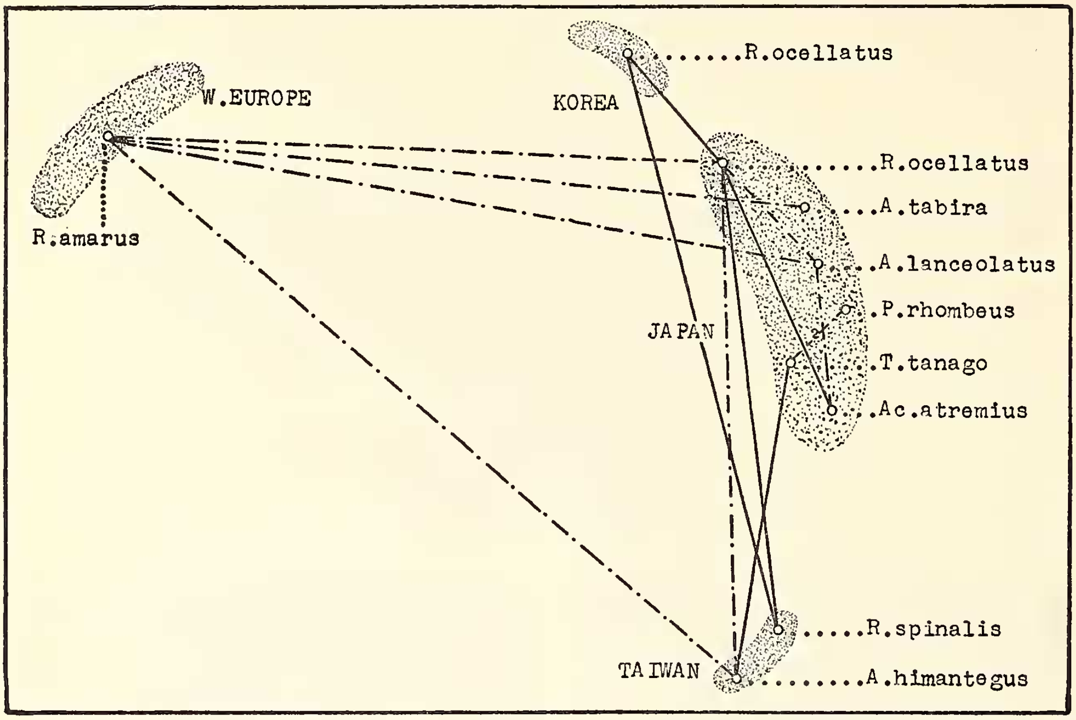
When comparing the phenotypes of these three strains, slight differences between both the Korean and the Japanese varieties of *R. ocellatus* are apparent, while the Taiwan species, *R. spinalis*, is fairly similar to both strains of *R. ocellatus*. In order to investigate whether the genomes of the three supposed subspecies would express themselves differently in the F_1 generations when crossed with a single species of bitterling belonging to another genus, we mated them with the Japanese species *Tanakia tanago*. In Table 1, the six possible breeding combinations have been designated 1 to 6.

Table 1.

	T. tanago ♀	*T. tanago* ♂
R. ocellatus (Japan) ♀		1
R. ocellatus (Japan) ♂	2	
R. ocellatus (Korea) ♀		3
R. ocellatus (Korea) ♂	4	
R. spinalis (Taiwan) ♀		5
R. spinalis (Taiwan) ♂	6	

Material and Method

All crossings were performed by means of artificial insemination. The number of larvae produced by the six crosses ranged from 8 to 20, but some died in the course of development as a result of infectious diseases. The following results are based on the hybrids that reached the adult stage.



TEXT-FIG. 1. Diagram showing the gametic affinities between a number of acheilognathine species from W. Europe, Korea, Japan and Taiwan, on the subspecies, species and generic levels, according to Duyvené de Wit [in press].

Results

1. *R. ocellatus* (Japan) ♀ × *Tanakia tanago* ♂

This combination yielded eight hybrids of different body size and shape. Two of them were large and fairly similar to the maternal species, *R. ocellatus* (Japan), four were small and showed greater similarity to the paternal species, *T. tanago,* while two were intermediate. All hybrids showed a male phenotype. During the spawning season they displayed full nuptial colors. Tubercles were present on the top of the snout. No milt production could be detected by stripping, however.

Representative specimens from the large and the small form of hybrid are illustrated in Figs. 3 and 4 respectively. The maternal species, *R. ocellatus* from Japan, is illustrated in Fig. 1 and the paternal species, *T. tanago,* in Fig. 2.

2. *T. tanago* ♀ × *R. ocellatus* (Japan) ♂

From this, the reciprocal combination, five hybrids were obtained. They also were not uniform in appearance. Three of them were large and in body size and shape tended to resemble the paternal species, *R. ocellatus* (Japan). The remaining two hybrids were medium-sized. One of them tended to resemble the maternal species, *T. tanago,* while the other was more similar to the paternal species, *R. ocellatus* (Japan). All hybrids showed a male phenotype. Nuptial colors and tubercles on top of the snout were present during the spawning season, but no milt could be obtained by stripping.

One of the large hybrids is illustrated in Fig. 7. A specimen of the maternal species, *T. tanago,* is illustrated in Fig. 5, and one of the paternal species, *R. ocellatus* from Japan, in Fig. 6.

It should be noted that the hybrids of the reciprocal combinations are different in general appearance.

3. *R. ocellatus* (Korea) ♀ × *T. tanago* ♂

From this combination six hybrids were obtained. They were relatively small and fairly uniform in size and body shape. In general appearance and with respect to their small size they were much more similar to the paternal species, *T. tanakia,* than to the maternal species, *R. ocellatus* (Korea). All hybrids showed a male phenotype. During the spawing season they displayed bright nuptial colors and tubercles were present on the top of the snout. Milt production could not be detected by means of stripping.

A representative hybrid specimen is illustrated in Fig. 9. The maternal species, *R. ocellatus* from Korea, is illustrated in Fig. 8.

4. *T. tanago* ♀ × *R. ocellatus* (Korea) ♂

From this cross five hybrids were obtained. Four of them were fairly large. With respect to body size and shape they tended to resemble the paternal species, *R. ocellatus* (Korea). One of these showed a coin-like body shape, an abnormality that is also sometimes encountered in the pure-bred strain of *R. ocellatus.* The remaining hybrid was much smaller than its brood mates. All hybrids showed a male phenotype. They displayed full nuptial colors during the spawning season and tubercles were present on the top of the snout. No milt production could be detected by stripping.

A representative hybrid specimen is illustrated in Fig. 11. The paternal species, *R. ocellatus* from Korea, is illustrated in Fig. 10.

In their general appearance the hybrids of the combinations 3 and 4 differ considerably from each other.

5. *R. spinalis* ♂ × *T. tanago* ♀

From this combination fourteen hybrids were obtained. With respect to body size they showed a gradual transition from large to very small. The largest specimens tended to resemble the maternal species, although the dorsal part of the body was less curved than in *R. spinalis.* The smaller ones were more similar to the paternal species, *T. tanago.* Except the three smallest specimens, which probably were neuters, the hybrids showed a male phenotype. During the spawning season they displayed full nuptial colors, and tubercles were present on the top of the snout. No milt could be obtained by stripping.

Representative specimens of the large and the small hybrids are illustrated in Fig. 13. The maternal species, *R. spinalis,* is illustrated in Fig. 12.

6. *T. tanago* ♀ × *R. spinalis* ♂

From this combination, five hybrids were obtained. They were fairly uniform in size and body shape and tended to resemble the paternal species in that the dorsal part of the body was curved as in *R. spinalis.* All of them showed a male phenotype. During the spawning season they displayed full nuptial colors. Tubercles were present on the top of the snout, but milt could not be obtained by stripping.

A representative specimen of the present hybrids is illustrated in Fig. 15. The paternal species, *R. spinalis,* is illustrated in Fig. 14.

The hybrids of combinations 5 and 6 show considerable difference in general appearance.

Discussion

The question whether the genomes of the three supposed geographical subspecies of *R. ocellatus* under investigation express themselves differently in the six kinds of hybrids that could be produced by crossing them with a single test species, *T. tanago,* must be answered in the affirmative.

In their general appearance the hybrids of the combinations 1 + 2, 3 + 4 and 5 + 6 appeared to differ considerably from one another. Moreover, the hybrids obtained from three hybrid combinations (1, 3 and 5) were different from their respective reciprocal ones (2, 4 and 6). Finally, the six separate F_1 generations showed no uniformity among their brood mates. These findings are in sharp contrast to the fact that the progenies obtained from *R. ocellatus* (Japan) × *R. ocellatus* (Korea), *R. ocellatus* (Japan) × *R. spinalis* (Taiwan) and *R. ocellatus* (Korea) × *R. spinalis* (Taiwan) were largely uniform in body form and size.

On the other hand, all present hybrid generations correspond in showing the male phenotype, by displaying nuptial colors during the spawning season and in the absence of milt production.

The lack of uniformity in the six F_1 generations probably points to incomplete genetic compatibility and presents an interesting problem for future karyological research.

The taxonomic status of the six kinds of hybrids under discussion here will be published separately.

Summary

1. Intergeneric hybrids have been obtained from the combinations *Rhodeus ocellatus* (Japanese strain) × *Tanakia tanago, Rhodeus ocellatus* (Korean strain) × *Tanakia tanago* and *Rhodeus spinalis* × *Tanakia tanago.*

2. All hybrids showed the male phenotype. During the spawning season they displayed full nuptial colors, but milt production could not be detected by means of stripping.

3. The six kinds of hybrids obtained from the three combinations and their reciprocals differed considerably from one another, and the variability among the individuals of the separate F_1 generations was conspicuous. This is interpreted as an expression of incomplete genetic compatibility between the respective male and female gametes.

Acknowledgements

The author is greatly indebted to Prof. Tokiharu Abe and Dr. Yoshitsugu Hirosaki for supplying the Japanese acheilognathine species, to Prof. Moon-ki Chyung and Mr. Kyun Hyun Kim for supplying the Korean acheilognathine species, to Professor Fah-hsuen for supplying the Taiwan acheilognathine species, to Mr. F. G. de Jardin and Mr. R. A. Cragg for providing photographs of the illustrated specimens, and to Mr. C. A. van Ee for the drawing of the diagram. The financial assistance of the Board of the University of the Orange Free State and of the Council for Scientific and Industrial Research of the Republic of South Africa is gratefully acknowledged.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Female specimen of *Rhodeus ocellatus* from Japan. Standard length 44 mm.

Fig. 2. Male specimen of *Tanakia tanago*. Standard length 42 mm.

Fig. 3. Specimen of a large hybrid obtained from the combination female *Rhodeus ocellatus* (Japan) × male *Tanakia tanago*. Standard length 61 mm.

Fig. 4. Specimen of a small hybrid obtained from the combination as indicated in the legend of Fig. 3. Standard length 37 mm.

Fig. 5. Female specimen of *Tanakia tanago*. Standard length 35 mm.

Fig. 6. Male specimen of *Rhodeus ocellatus* from Japan. Standard length 69 mm.

Fig. 7. Specimen of a hybrid obtained from the combination female *Tanakia tanago* × male *Rhodeus ocellatus* (Japan). Standard length 60 mm.

Fig. 8. Female specimen of *Rhodeus ocellatus* from Korea. Standard length 44 mm.

Fig. 9. Specimen of a hybrid obtained from the combination female *Rhodeus ocellatus* (Korea) × male *Tanakia tanago*. Standard length 37 mm.

Plate II

Fig. 10. Male specimen of *Rhodeus ocellatus* from Korea. Standard length 61 mm.

Fig. 11. Specimen of a hybrid obtained from the combination female *Tanakia tanago* × male *Rhodeus ocellatus* (Korea). Standard length 51 mm.

Fig. 12. Female specimen of *Rhodeus spinalis*. Standard length 39 mm.

Fig. 13. Specimen of a large hybrid obtained from the combination female *Rhodeus spinalis* × male *Tanakia tanago*. Standard length 54 mm. Left corner: specimen of the very small hybrid form. Standard length 25 mm.

Fig. 14. Male specimen of *Rhodeus spinalis*. Standard length 46 mm.

Fig. 15. Specimen of a hybrid obtained from the combination female *Tanakia tanago* × male *Rhodeus spinalis*. Standard length 45 mm.